Different Types of Australia Antigen Detected by Radioimmunoassay and Immunoelectrosyneresis

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MATSUDA, S., SUKENO, N. and ISHIDA, N. Different Types of Australia Antigen Detected by Radioimmunoassay and Immunoelectrosyneresis. Tohoku J. exp. Med., 1972, 108 (1), 95-96 — A mixed human serum containing Australia (Au) antigen was fractionated by a discontinuous sucrose gradient centrifugation. When Au antigen in each fraction was assayed by radioimmunoassay (RIA) and immunoelectrosyneresis (IES), the peaks in titer of both assay systems appeared in different fraction. The RIA peak fraction contained spherical particles with dense core and tubular forms. ———— hepatitis B; Australia antigen; radioimmunoassay

For detection of Au antigen, many methods have been proposed to increase the sensitivity. The highest sensitivity of the RIA, which was introduced by Abbott Laboratories as Ausria-125 kit, has been reported (Matsuda et al. 1972a) and this method has been used for detecting antigens in amniotic fluid (Matsuda et al. 1972b) and saliva (Matsuda et al., Lancet, in preparation). However, it is not yet established whether or not these assay systems are detecting the same antigenic sites or types in the Au positive specimens. The present report is concerned with the different antigenicity detected by the two methods.

Human sera found to be Au positive by IES were obtained from 3 healthy adults and pooled to 200 ml. The GOT value of these sera were normal. The pool was centrifuged at 68,000 g for 20 hours by a Hitachi 40P ultracentrifuge after adjusting pH to 4.0. The obtained pellet was dispersed in 20 ml of 0.01 M veronal buffer (pH 8.6) and treated with Pronase (Kaken Kagaku Ltd., Tokyo) at a concentration of 500 μg/ml, followed by the incubation at 37°C for half an hour. Finally the enzyme-treated preparation was layered on a discontinuous gradient of sucrose (40%, 60%) and centrifuged at 68,000 g for 20 hours. After centrifugation, the sample was divided into sixteen fractions by bottom-drip method, and Au antigen in each fraction was assayed by the RIA and IES as previously reported (Matsuda et al. 1972a). Fig. 1 illustrates that the highest cpm peak detected by the RIA (4,755 cpm) is in Fraction 10 at sucrose concentration of 16.3%, whereas the highest dilution titer peak by IES (× 32) appeared in Fraction 14 at sucrose concentration of 13.9%. In Fraction 10, where the highest RIA titer is found, the titer of IES was 1/32 of that in Fraction 14.

Preliminary electron microscopic studies conducted by J. Yamaguchi and Y. Amano revealed different particle distributions in two peaks. The IES peak fraction showed uniform distribution of spherical Au particles of regular size, whereas the RIA peak fraction revealed the presence of small and large spherical particles containing dense core materials and short tubular forms. Some of these spherical particles are related to Dane particles (Dane, 1970).

For the explanation of such selected detection ability of the RIA kit, the guinea pig

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antibody coated to the kit tubes might be different from the human serum used in IES in a sense that the guinea pig antibody was directed to different antigenic sites of hepatitis B virus or Australia antigen. Although the repeated fractionation trials with different serum sources reproduced the same two peaks, further systematic study is required to establish the significance of this observation.

References